Gastrointestinal morphology and absorption of monosaccharides in fowls conditioned to different types and levels of dietary fibre

BY C. J. SAVORY

AFRC Institute of Animal Physiology and Genetics Research, Roslin, Midlothian EH25 9PS

(*Received 31 August 1990 – Accepted 8 May 1991*)

To test a possible influence of dietary fibre on intestinal sugar uptake, rates of absorption of 10 mM-D-[U-^{14}C] glucose and 10 mM-D-[U-^{14}C] xylose were measured in either jejunum or (distal) caecum, by in vivo lumen perfusion, in immature female fowls preconditioned to a standard diet containing (g/kg) either 0, 100, 200 or 400 added dried grass, 200 powdered cellulose, or 200 grass with a polysaccharidase enzyme supplement. When birds were killed after perfusion, dimensions of (unperfused) parts of their alimentary tracts were determined, and recoveries of ^{14}C radioactivity in some body tissues were compared with measured activities absorbed. On average, absorption of glucose was 1·9 and 1·2 times faster than xylose in jejunum and caecum respectively, although these differences varied with dietary treatment and order of perfusion. Increasing grass in the diet caused significant changes in xylose absorption rate in both jejunum and caecum, but only when it was perfused before glucose. With any one sugar and intestinal segment, mean rates of absorption were correlated positively with corresponding mean rates of fluid loss from perfusate. Although their influence on sugar absorption was not well defined, the dietary fibre treatments had more pronounced effects on gross dimensions of parts of the alimentary tract and, hence, potentially on total rates of absorption. Compared with the basal diet, addition of 100, 200 or 400 g grass/kg or 200 g cellulose/kg caused significant increases in small intestine length while 200 g grass/kg with supplementary enzyme did not, and combined caecal length increased with the 400 g grass/kg and the supplementary enzyme treatments. Absorbed ^{14}C activity was recovered in plasma after jejunal perfusions but not caecal ones, whereas it was recovered in liver and in the flushed perfused segment after both types of perfusion. Since there was overlap in absorption rates between jejunum and caecum, this result suggests that the liver may be able to distinguish and treat differently compounds absorbed in the two regions.

Dietary fibre: Monosaccharide uptake: Gastrointestinal morphology: Fowl

As part of an investigation into possible benefits of supplementing non-ruminant feedstuffs with exogenous polysaccharidase enzymes, rates of absorption of four constituent sugars of plant cell walls (glucose, galactose, xylose, arabinose) were compared in jejunum, ileum and caecum in the fowl (Savory & Mitchell, 1991). Evidence was obtained that patterns of monosaccharide absorption were influenced by the composition of the diet to which birds were conditioned before being tested. Specifically, with a standard (ST) mash diet to which 200 g grass/kg was added, the absorption rate of hexose (glucose, galactose) was 30% higher in the jejunum and 29% lower in the caecum than with the basal diet alone. This was not conclusive, however, because the diet comparison involved two groups of birds which were tested at different times. To investigate any such effects of dietary fibre in more detail, therefore, another experiment is reported here in which birds were conditioned to the same ST diet as before, diluted with either 0, 100, 200, 400 g grass/kg, 200 g grass/kg with added enzyme, or 200 g cellulose/kg. Unlike the first experiment (Savory & Mitchell, 1991), in which birds were each tested with all four sugars, here they were tested with one hexose (D-glucose) and one pentose (D-xylose) only, in either jejunum or caecum.

Although effects of dietary fibre level on sugar absorption have not been studied before in the fowl, it has been shown that rates of intestinal glucose and fructose absorption were higher in chicks fed on a carbohydrate-free diet than in those fed on diets rich in carbohydrate (Gauthier *et al.* 1979). It has also been shown that deprivation of food for 3 d caused significant changes in kinetic characteristics of (electrogenic) glucose and galactose transfer in jejunum and ileum of fowls (Levin & Mitchell, 1982). In rats, intestinal zinc transfer was influenced by manipulation of dietary fibre (Seal & Mathers, 1989), and absorption of amino acids by manipulation of protein intake (Lis *et al.* 1972).

In addition to sugar transfer, dimensions of different parts of the alimentary tract were also measured in the present experiment in order to assess broader aspects of gastrointestinal (GI) adaptation to dietary fibre and to relate these to observed rates of absorption. Changes in GI morphology associated with variation in dietary fibre have been reported in wild gallinaceous birds (Leopold, 1953; Lewin, 1963; Pendergast & Boag, 1973; Moss, 1974) and captive ones (Moss, 1972; Savory & Gentle, 1976*a*, *b*), but not, so far, in the fowl. Finally, since the method used here for measuring rates of intestinal absorption (Levin *et al.* 1983) is based on in vivo lumen perfusion of ^{14}C-labelled compounds, an attempt was made to validate the technique by relating levels of ^{14}C radioactivity absorbed during perfusion to those recovered in plasma and liver at the end of the test.

MATERIALS AND METHODS

Subjects and diets

The subjects were ninety-six immature female medium-hybrid (Rhode Island Red × Light Sussex) fowls, 12–14 weeks old and weighing 1·03–1·50 kg. Separate hatches were used to ensure that birds were all about the same age when tested (Table 3), the whole experiment taking about 3 months to complete. Within each hatch, equal numbers of birds were conditioned to each of the six diets in Table 1 for at least 3 weeks before testing, which was the time taken for complete adaptation of GI morphology after manipulation of dietary fibre content in Japanese quail (*Coturnix coturnix japonica*; Savory & Gentle, 1976*b*). All birds were kept on a 14 h photoperiod at about 20° and had *ad lib.* access to food and water until the time of testing.

Experimental design

With each dietary treatment, half the sixteen birds were tested in the jejunum and half in the (left) caecum. With each intestinal segment, half the eight birds were tested with glucose and then xylose, and half in the reverse order. There were, thus, six diets × two segments × two orders × four birds, and within each hatch different treatments were tested in random order.

Perfusion procedure for measuring rates of absorption

Solutions of 10 mM-D-glucose and D-xylose were prepared in bicarbonate–saline (9 g sodium chloride/l) buffer (Krebs & Henseleit, 1932), with each containing enough of the respective sugar in [U-^{14}]-labelled form (Amersham International) to give a specific activity of 0·4 μCi per 50 ml, and 100 mg fluorescein isothiocyanate (FITC) dextran (Sigma) per 250 ml for measuring fluid loss during perfusion. Reasons for choosing the 10 mM concentration and FITC dextran marker were given previously (Savory & Mitchell, 1991). Up to three birds were tested per day according to the perfusion schedule in Table 2. Methods for inducing and maintaining anaesthesia, exposing and cannulating segments of jejunum and caecum, maintaining birds' body heat and the pH of perfusates, measuring fluid loss and ^{14}C radioactivity, and calculating rates of absorption of the glucose and xylose were all as described previously (Savory & Mitchell, 1991).

Table 1. *Composition of test diets (g/kg)*

Diet...	0G (ST)	10G (ST + 100 g grass/kg)	20G (ST + 200 g grass/kg)	40G (ST + 400 g grass/kg)	20C (ST + 200 g cellulose/kg)	20G/E (ST + 200 g grass/kg + 10 g FENZ/kg)
Barley	300	270	240	180	240	237·6
Maize	200	180	160	120	160	158·4
Wheat	280	252	224	168	224	221·8
Grass	50	145	240	430	40	237·6
Soya bean	100	90	80	60	80	79·2
Herring	35	31·5	28	21	28	27·7
Limestone	10	9	8	6	8	7·9
Dicalcium phosphate	17·5	15·8	14	10·5	14	13·9
Cellulose (CEPO)	—	—	—	—	200	—
Salt	2·5	2·3	2	1·5	2	2·0
Vitamin mix	2·5	2·3	2	1·5	2	2·0
Mineral mix	2·5	2·3	2	1·5	2	2·0
Enzyme (FENZ)*	—	—	—	—	—	10·0
NDF†	52	89‡	125	198‡	> 125‡	124‡
Crude protein (nitrogen × 6·25)†	148	154‡	160	172‡	123‡	161‡
AME† (MJ/kg)	11·2	10·8‡	10·4	9·6‡	9·3‡	10·3‡

NDF, neutral-detergent fibre; AME, apparent metabolizable energy; ST, basal diet.

* A composite enzyme preparation containing different polysaccharidase activities, details of which are confidential.

† NDF, crude protein and AME were measured with the 0G and 20G diets (Savory & Mitchell, 1991).

‡ These values were extrapolated from those measured with the 0G and 20G diets.

Table 2. *Perfusion schedule*

1. Anaesthetize bird with halothane (Fluothane; ICI; 30 ml/l, 2 l/min)
2. Expose intestinal segment, clean thoroughly with saline (9 g sodium chloride/l), implant cannulas and reposition segment in abdomen
3. Fill system from 50 ml Krebs Ringer (buffer) solution (flow-rate 14·5 ml/min, about 2 min)
4. Perfuse with Krebs Ringer (buffer) solution for 5 min (flow-rate 4 ml/min)
5. Empty system (flow-rate 14·5 ml/min, about 2 min)
6. Fill system from 50 ml sugar 1
7. Perfusion with sugar 1 for 2 min (flow-rate 4 ml/min)
8. Empty system and remove two 1 ml portions
9. Fill system from 50 ml sugar 1
10. Perfuse with sugar 1 for 15 min (flow-rate 4 ml/min). Measure breathing rate and body temperature (with rectal probe)
11. Empty system and remove two 1 ml portions
12. Return to step 3 and repeat (steps 3–11) for sugar 2 (total time 65–70 min for complete schedule)

At the end of testing, remove 1 ml blood from wing vein into a heparinized syringe. Fill system with Krebs and flush segment (flow-rate 4 ml/min) for 5–10 min. Empty system, kill bird with 2 ml sodium pentobarbitone (Sagital, May and Baker) injected into wing vein. Remove perfused segment and measure immediately to the nearest 0·1 cm (in a vertical position with a 10 g weight attached to bottom end). Measure 'wet' weight before and after removing 0·25 g portion for radioactivity (disintegrations/min) count, and dry weight of remainder after oven-drying at 80° for 48 h. Remove about 4 g liver for radioactivity count, prepare portion of intestine for villi measurement, measure gut dimensions.

Recovery of absorbed ^{14}C activity

In order to determine whether measured absorption of U-^{14}C-labelled sugars in jejunum and caecum was reflected by ^{14}C activity in circulating plasma, 1 ml blood was removed by wing vein from each bird at the end of testing (Table 2). This was centrifuged (2500 ***g***, 5 min), 0·5 ml plasma was added to 4·5 ml Optiphase X (Pharmacia Ltd) and its radioactivity measured in a liquid-scintillation counter (LKB Wallac Rackbeta). The results of these measurements were anomalous, so radioactivity in the liver was also measured after removing a portion from the freshly killed bird. Later it was considered that some absorbed ^{14}C activity might be retained in enterocytes on the villi in perfused segments so, after thorough flushing with Krebs' bicarbonate buffer, a small portion of the (fresh) segment was also removed. Liver and intestinal portions were kept deep-frozen until the end of the experiment, when they were all analysed at the same time. A weighed amount (0·15–0·25 g wet weight) of each was dissolved completely in 2·0 ml Optisolve (Pharmacia Ltd; in about 3 d), and then two 0·5 ml portions of this were each added to 4·5 ml Optiscint T (Pharmacia Ltd) to measure radioactivity. Since the collections of liver and (especially) intestinal segments commenced after the start of the experiment, their total sample sizes are less than ninety-six (Table 9).

GI morphology measurements

After birds were killed at the end of testing, measurements were made of lengths and weights of perfused intestinal segments in all birds (Table 2), of combined caeca length in birds with perfused jejunum, and of empty gizzard weight and lengths of duodenum, jejunum, ileum and colo-rectum in birds with perfused caecum. With jejunum perfusions, a segment (1 cm) of jejunum was removed immediately anterior to the perfused section, and with caecal perfusions, a segment (1 cm) was removed from the unperfused (right) caecum, 2 cm from its distal end. These segments were opened out, cleaned with isotonic saline, pinned on bits of balsa wood with mucosa uppermost, and then inverted in buffered neutral formalin for 1 week to be fixed. They were then sectioned and stained with haematoxylin and eosin, and lengths of complete villi (ten with caecum but usually fewer with jejunum) were measured to the nearest 0·05 mm with a binocular microscope and graticule scale, to obtain mean villus length for each segment.

Food intake and digestibility measurements

Mean daily food intakes were obtained from six birds on each dietary treatment by measuring weights of food eaten over periods of 4–10 d, when they were housed individually and had been fed on their respective diets for at least 2 weeks. To measure digestibilities, four (conditioned) birds on each treatment were fed their respective diet supplemented with 4 g titanium dioxide/kg for 3 d, their droppings trays were cleaned at the start of the third day, and all excreta produced by each bird on the third day were collected. Excreta and a sample of each marked food were oven-dried and analysed for TiO_2, and apparent digestibility of the food was calculated for each bird (Peddie *et al.* 1982).

Statistical analysis

Age and body-weight at testing, daily food intake, apparent digestibility and GI morphology measurements were each compared between dietary treatments by one-way ANOVA (Tables 3, 4). Significance of effects of diet, intestinal segment, order of perfusion and interactions on absorption variables was obtained from three-way ANOVA; and in addition, the effect of 'grass linear dose' (diets 0G, 10G, 20G, 40G) was tested by regression analysis (Table 5). Levels of ^{14}C radioactivity that were absorbed during perfusion and

Table 3. *Mean age and body-weight at testing, daily intake and apparent digestibility of test diets, for birds conditioned to the diets for at least 3 weeks*

Diet*...	*n*	0G	10G	20G	SED	Statistical significance of variance ratio
Age (d)	16	90·8	92·1	92·0	1·9	NS
Body-wt (kg)	16	1·26	1·27	1·26	0·04	NS
Daily intake (g)	6	92·5[a]	94·0[a]	96·8[a]	3·8	$P = 0·042$
Digestibility (%)	4	70·2[a]	66·4[b]	58·8[c]	1·4	$P < 0·001$

Diet*...	*n*	40G	20C	20G/E	SED	Statistical significance of variance ratio
Age (d)	16	92·0	92·1	92·3	1·9	NS
Body-wt (kg)	16	1·22	1·22	1·32	0·04	NS
Daily intake (g)	6	97·0[a]	104·8[b]	95·7[a]	3·8	$P = 0·042$
Digestibility (%)	4	56·5[c]	58·3[c]	63·0[d]	1·4	$P < 0·001$

0G, basal diet; 10G, 20G, 40G, basal diet with 100, 200 and 400 g grass/kg; 20C, basal diet with 200 g cellulose/kg; 20G/E, basal diet with 200 g grass/kg and 10 g enzyme preparation/kg.

n, sample size with each diet; SED, standard error of the difference between means; NS, not significant ($P > 0·05$);

[a, b, c, d] Means with the same superscript letter were not significantly different.

* For details of diets, see Table 1.

recovered in plasma, liver and intestinal segment were compared between jejunum- and caecum-perfused birds by *t* test (Table 9), and also correlated with each other within the two groups of birds (Table 10).

RESULTS

Food intake and digestibility

Body-weight at the time of testing was greatest with the enzyme-supplemented diet (20G/E), but did not differ significantly ($P > 0·05$) between dietary treatments (Table 3). Daily food intake was significantly higher with diet 20C than with the other diets. Addition of grass or cellulose caused reductions in apparent digestibility compared with the basal diet (0G), but the enzyme supplement (diet 20G/E) caused a significant 7% improvement in digestibility compared with the unsupplemented diet 20G (Table 3).

GI morphology

Empty gizzard weight, jejunum length, and jejunal and caecal villus lengths did not differ significantly between treatments (Table 4). Lengths of duodenum, ileum and total small intestine, however, all increased with addition of grass or cellulose, except when enzyme was added as well (diet 20G/E). Combined caeca were longer with diets 40G and 20G/E than with other treatments, while the colo-rectum was shortest with the basal diet (0G).

Rates of sugar absorption

Perfused segments of intestine did not differ significantly between dietary treatments in either length (overall means: jejunum 12·9 cm, caecum 8·3 cm) or dry weight (jejunum 0·88 g, caecum 0·35 g).

On average, glucose absorption was 1·9 and 1·2 times faster than xylose absorption in jejunum and caecum respectively, although these differences varied with dietary treatment

Table 4. *Mean dimensions of (unperfused) parts of the alimentary tract (after testing) in birds conditioned to the test diets for at least 3 weeks*

(Mean values for eight birds per dietary treatment)

Diet...	0G	10G	20G	40G	20C	20G/E	SED	Statistical significance of variance ratio
Empty gizzard (g)	27·5	28·3	29·6	28·9	27·9	31·6	2·0	NS
Duodenum (cm)	22·9^{a}	24·9^{bc}	25·4^{c}	25·0^{bc}	24·4^{abc}	23·2^{ab}	0·9	$P = 0·036$
Jejunum (cm)	53·2	57·0	59·1	56·1	57·5	55·6	2·3	NS
Ileum (cm)	49·4^{a}	55·6^{b}	58·7^{b}	56·8^{b}	56·4^{b}	53·4^{ab}	2·7	$P = 0·026$
Total small intestine (cm)	125·5^{a}	137·4^{bc}	143·2^{c}	137·9^{bc}	138·2^{bc}	132·2^{ab}	5·0	$P = 0·021$
Combined caeca (cm)	37·1^{a}	36·9^{a}	37·7^{a}	40·2^{b}	36·0^{a}	40·3^{b}	1·1	$P = 0·001$
Colo-rectum (cm)	8·5^{a}	9·2^{b}	9·8^{bc}	9·8^{bc}	9·6^{bc}	10 ·0^{c}	0·3	$P < 0·001$
Jejunal villi (mm)	1·19	1·16	1·28	1·12	1·19	1·16	0·07	NS
Caecal villi (mm)	0·22	0·24	0·22	0·23	0·23	0·22	0·02	NS

$^{a, b, c}$ Means with the same superscript letter were not significantly different.

SED, standard error of difference between means; NS, not significant ($P > 0·05$); 0G, basal diet; 10G, 20G, 40G, basal diet with 100, 200 and 400 g grass/kg; 20C, basal diet with 200 g cellulose/kg; 20G/E, basal diet with 200 g grass/kg and 10 g enzyme preparation/kg.

* For details of diets, see Table 1.

Table 5. *Significance of effects of diet (six diets), intestinal segment (jejunum, caecum) and order of perfusion (glucose–xylose, xylose–glucose) on absorption variables, from three-way ANOVA (variance ratios with 72 residual and 95 total df)*

Effect	Absorption				Fluid loss	
	Glucose (/cm)	Xylose (/cm)	Glucose (/g)	Xylose (/g)	Glucose (/cm)	Xylose (/cm)
Diet	—	—	—	—	—	**
(Grass linear dose)	—	—	—	—	—	**
Segment	—	*	***	***	**	**
Order	***	*	***	*	—	—
Diet/segment	—	—	—	—	—	**
(Dose/segment)	—	***	—	**	—	***
Diet/order	—	—	—	—	—	—
(Dose/order)	*	*	—	*	**	***
Segment	—	**	*	**	—	**
Diet/segment/order	—	—	—	—	—	*
(Dose/segment/order)	—	**	—	*	—	***

Dose, grass linear dose, regression *v.* diets 0G, 10G, 20G, 40G.
* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$, all other variance ratios not significant ($P > 0{\cdot}05$).

and order of perfusion (Tables 6, 7). On a unit length basis, glucose absorption was affected significantly by order of perfusion and by a dietary grass (linear) dose × order interaction (Table 5). Thus, in both jejunum and caecum, glucose absorption was faster when it was perfused first, and this difference between first and second perfusions tended to decline with increasing grass in the diet (Table 6). With xylose absorption there were six significant effects, four of which were interactions (Table 5). Thus, there were overall tendencies for xylose absorption to be faster when perfused first, and faster in the caecum than jejunum. However, these effects were due mainly to very high rates of absorption when xylose was perfused first in the caecum with diets 0G and 10G, and there was no consistent effect of order of perfusion in the jejunum (Table 6). Also, with increasing grass in the diet, xylose absorption rates during the first perfusion increased in jejunum but decreased in caecum.

Because perfused segments weighed less per unit length with caecum (overall mean 42 mg/cm) than jejunum (69 mg/cm), reflecting longer villi (Table 4) and thicker muscle layers (Gentle & Savory, 1975) with the latter, absorption rates in the caecum were proportionately greater per unit weight than per unit length. Thus, on a dry-weight basis, absorption rates of both glucose and xylose were significantly higher in the caecum than jejunum (Tables 5 and 7). With glucose, the effect of order of perfusion, which was greater in the caecum than jejunum, was significant as before, but the effect of the grass dose × order interaction was not. With xylose, significant effects were the same as on a length basis, with the greatest influence coming from the very high values when it was perfused first in the caecum with diets 0G and 10G.

Rates of fluid transfer

Overall mean rates of fluid loss from perfusate across intestinal epithelium did not differ between glucose and xylose perfusions in either jejunum or caecum (Table 8). With both sugars, however, fluid loss was greater in the caecum than in the jejunum (Tables 5 and 8), reflecting the fact that the hind-gut is the main site of water resorption (Thomas & Skadhauge, 1988). When glucose was perfused first, the associated fluid loss in both jejunum and caecum tended to decline with increasing grass in the diet. This was also true

Table 6. *Mean rates of absorption of glucose and xylose (10 mM solutions) in the jejunum and caecum of birds conditioned to six test diets (nmol/min per cm)*

Sugar...	Glucose (SED 73)				Xylose (SED 64)			
Segment...	Jejunum		Caecum		Jejunum		Caecum	
Order*...	1	2	1	2	1	2	1	2
Diet								
0G	333	183	318	164	91	159	450	95
10G	302	175	373	164	128	89	269	137
20G	280	202	237	129	164	197	187	134
40G	284	308	277	202	164	188	121	120
20C	238	230	266	216	157	156	206	195
20G/E	373	290	195	218	81	135	164	133
Mean (*n* 24)	302	231	278	182	131	154	233	136

SED, standard error of the difference between means (*n* 4); 0G, basal diet; 10G, 20G, 40G, basal diet with 100, 200 and 400 g grass/kg; 20C, basal diet with 200 g cellulose/kg; 20G/E, basal diet with 200 g grass/kg and 10 g enzyme preparation/kg.

* The sugar concerned was perfused first or second.

when xylose was perfused first, but in the caecum only, where the effect was very marked. With xylose perfusions, fluid loss in the caecum was greater ($P < 0{\cdot}05$) with diet 20C than diet 20G. With any one sugar and segment, mean fluid loss values in Table 8 were correlated positively with corresponding mean absorption rates in Tables 6 and 7 ($P < 0{\cdot}01$ or $0{\cdot}001$, n 12).

Recovery of absorbed ^{14}C activity

There were no significant effects of dietary treatment or order of perfusion on recovery of ^{14}C activity in plasma, liver or perfused intestinal segment, so values from all treatments were combined for comparing jejunum and caecum.

Total radioactivity absorbed, and that absorbed with the second sugar only, were greater with jejunum than caecum perfusions (Table 9), mainly because of the difference in length of perfused segment (see overall means, p. 81). Radioactivity (disintegrations/min; dpm) in plasma after jejunum perfusions ranged from 58 to 171, but those after caecum perfusions were nearly all about background (radiation) level (12 dpm), with the highest being 32 dpm. Thus, absorbed ^{14}C activity was recovered in plasma after perfusions in the jejunum but not in the caecum. Activity recovered in the liver, however, did not differ significantly between jejunum and caecum perfusions (although the mean was 11% higher with the caecum, Table 9), thus validating measured sugar absorption from the caecum. Although activity recovered per gram of perfused segment did not differ significantly between jejunum and caecum, this was because of one exceptionally high radioactivity value (11 290 dpm) with the jejunum, which caused its larger standard error (Table 9). If this value is ignored, then mean activity recovered in jejunum was higher ($P < 0{\cdot}05$), presumably reflecting greater surface area on its longer villi (Table 4) for potential U-^{14}C-labelled sugar retention.

^{14}C activity in plasma reflected total activity absorbed, and that absorbed with the second sugar only, with jejunum but not caecum perfusions (Table 10). Instead, ^{14}C activity absorbed in the caecum was correlated negatively with that recovered in liver, and recovery in perfused caecum was also correlated negatively with that in liver. Multiple regression analyses confirmed that these were the only significant relationships.

Table 7. *Mean rates of absorption of glucose and xylose (10 mM solutions) in the jejunum and caecum of birds conditioned to six test diets (nmol/min per g dry wt)*

Sugar...	Glucose (SED 1494)				Xylose (SED 1496)			
Segment...	Jejunum		Caecum		Jejunum		Caecum	
Order*...	1	2	1	2	1	2	1	2
Diet								
0G	4195	2847	7249	3705	1390	2068	10167	2454
10G	4161	2440	8927	3654	1773	1206	6114	3358
20G	4216	2895	5384	3316	2180	2961	4684	3008
40G	4347	4520	8093	5462	2455	2780	3132	3550
20C	3683	3973	6243	4875	2609	2329	4989	4544
20G/E	5465	4659	4820	4770	1325	1957	3951	3270
Mean (*n* 24)	4345	3556	6786	4297	1955	2217	5506	3364

SED, standard error of the difference between means (*n* 4); 0G, basal diet; 10G, 20G, 40G, basal diet with 100, 200 and 400 g grass/kg; 20C, basal diet with 200 g cellulose/kg; 20G/E, basal diet with 200 g grass/kg and 10 g enzyme preparation/kg.

* The sugar concerned was perfused first or second.

Table 8. *Mean rates of fluid loss from 10 mM solutions of glucose and xylose in the jejunum and caecum of birds conditioned to six test diets (μl/min per cm)*

Sugar...	Glucose (SED 6·6)				Xylose (SED 5·1)			
Segment...	Jejunum		Caecum		Jejunum		Caecum	
Order*...	1	2	1	2	1	2	1	2
Diet								
0G	11·7	7·8	21·7	11·8	6·7	11·0	42·1	9·2
10G	10·5	9·3	34·4	12·5	12·8	6·9	16·3	15·0
20G	8·3	7·9	16·7	8·5	13·6	13·0	13·4	9·3
40G	8·9	16·9	11·0	19·5	9·8	11·5	4·7	14·0
20C	6·4	8·0	25·1	7·0	13·7	12·4	20·0	21·1
20G/E	14·6	13·1	9·9	18·1	5·0	14·4	14·2	3·2
Mean (*n* 24)	10·1	10·5	19·8	12·9	10·3	11·5	18·5	12·0

SED, standard error of the difference between means (*n* 4); 0G, basal diet; 10G, 20G, 40G, basal diet with 100, 200 and 400 g grass/kg; 20C, basal diet with 200 g cellulose/kg; 20G/E, basal diet with 200 g grass/kg and 10 g enzyme preparation/kg.

* The sugar concerned was perfused first or second.

The differences between jejunum and caecum perfusions, in ^{14}C recovery in plasma (Table 9) and in recovery interrelationships (Table 10), cannot be accounted for by a fundamental difference in absorption rate since there was plenty of overlap between the two regions (ranges in total radioactivity absorbed were $28–294 \times 10^3$ and $18–199 \times 10^3$ dpm in jejunum and caecum respectively). A possible explanation that was tested was that in caecal perfusions sugars might have been converted to volatile fatty acids (VFA) before being absorbed by caecal bacteria left adhering to villi, and that these absorbed VFA did not pass from the liver into the circulation. However, when VFA were extracted by steam distillation from the homogenized livers of all birds with the diet 20G/E pretreatment (Table 1), there was no difference between the minimal radioactivity levels of distillates from jejunum- and

Table 9. *Mean ^{14}C radioactivity (disintegrations/min) absorbed during perfusion (total from both sugars, and from second sugar (sugar 2) only), and its recovery from plasma, liver and flushed perfused intestinal segment**

(Mean values with their standard errors)

	Jejunum			Caecum			Statistical significance of difference
	n	Mean	SE	*n*	Mean	SE	
Total ($\times 10^3$)	48	140·8	8·4	48	91·5	5·8	$P < 0·001$
Sugar 2 ($\times 10^3$)	48	66·2	4·8	48	36·1	3·2	$P < 0·001$
Plasma (per 0·5 ml)	48	110·5	4·7	48	13·2	0·7	$P < 0·001$
Liver (per g wet wt)	43	4303	480	43	4759	444	NS
Segment (per g wet wt)	24	1845	429	25	1067	79	NS

NS, not significant ($P > 0·05$).
* For details of procedures, see Table 2 and pp. 78–80.

Table 10. *Cross correlations* (r) *between ^{14}C radioactivity (disintegrations/min) absorbed (total from both sugars, and from second sugar (sugar 2) only) and that recovered from plasma, liver and flushed perfused intestinal segment*

	Sugar 2	Plasma	Liver	Segment
Jejunum				
n	48	48	43	24
Total	0·57***	0·39**	−0·25	−0·32
Sugar 2	—	0·39**	−0·07	−0·15
Plasma	—	—	0·02	−0·34
Liver	—	—	—	0·23
Caecum				
n	48	48	43	25
Total	0·72***	−0·02	−0·36*	−0·13
Sugar 2	—	0·14	−0·29*	−0·13
Plasma	—	—	−0·03	0·28
Liver	—	—	—	−0·38*

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$, all other correlation coefficients not significant ($P > 0·05$).

caecum-perfused birds. In fact, such an explanation seems unlikely anyway. This is because VFA appear to be absorbed entirely by passive diffusion (Sudo & Duke, 1980), and so their absorption should be fastest when their concentrations in plasma are lowest. Their concentrations in portal blood reflect those in caecal contents (Cheng *et al.* 1987), which in turn declined with the increasing dietary grass levels used in the present study (Savory & Knox, 1991); yet absorption rates in caecum declined with increasing grass (Tables 6 and 7, first perfusion), when presumably VFA absorption rates should have increased. It appears, therefore, that sugars absorbed in caecum are treated differently, presumably in the liver, to those absorbed in jejunum.

DISCUSSION

Just as rates of intestinal sugar absorption in the fowl were found to be affected by dietary carbohydrate treatments in growing chicks (Gauthier *et al.* 1979), so the results of the present experiment indicate that they are also influenced by different levels of dietary fibre. The effects of dietary treatment observed here agree with those in the previous experiment

of Savory & Mitchell (1991; see p. 77) in one respect, in that there was a decline in hexose absorption rate in the caecum between diets 0G and 20G (Tables 6 and 7). There was no evidence here, however, of the increase in the hexose absorption rate in the jejunum seen previously between the same two diets. Nor were effects of dietary treatment here defined well enough to give any clear indication of their causation. Since villus lengths in jejunum and caecum did not differ between dietary treatments (Table 4), it seems unlikely that there would have been variation in absorptive surface area per unit length or weight of intestine. Systematic variation in a passive component of absorption, dependent on a downhill concentration gradient, also seems unlikely, especially with glucose which is normally regulated within narrow limits in the blood of fowls (Savory, 1987).

Rates of sugar absorption were closely correlated with rates of fluid loss from perfusate (Tables 6, 7 and 8), perhaps due to combined effects of osmotic influences and solvent drag (Crane, 1974; Pappenheimer & Reiss, 1987), as discussed by Savory & Mitchell (1991). It is conceivable, therefore, that dietary effects on nutrient uptake could be at least partly indirect if the treatments themselves affect patterns of fluid transfer. However, no further information on fluid balance was obtained in the present study. The tendency for rates of sugar absorption and fluid loss in the caecum to decline after the first perfusion (Tables 6, 7 and 8), as also found in the previous experiments (Savory & Mitchell, 1991), may possibly reflect suppression of an aldosterone-controlled fluid transfer mechanism in this (paired) organ, which is one of the main sites of water resorption in birds (Thomas & Skadhauge, 1988).

It is also conceivable that dietary effects on sugar absorption could be due to variation in levels of constituents other than fibre, or to variation in food intake. The dietary treatments here, however, differed much more in fibre content than in either their crude protein or metabolizable energy contents (Table 1), and diet 20C treatment was the only one where food intake increased significantly (Table 3). Also, grass is sufficient in most vitamins and minerals (Bolton & Blair, 1974), so it seems unlikely that a deficiency in one of these could have caused the observed effects of increasing grass. Nevertheless, food intake might be important, because it, rather than fibre *per se*, was concluded to be the main reason for gross changes in GI morphology in Japanese quail (Savory & Gentle, 1976*a*).

Although their influence on absorption was not well defined, the present dietary treatments had more pronounced effects on gross dimensions of the duodenum, ileum, (total small intestine), caeca and colo-rectum (Table 4) and, hence, (presumably) on total surface area of mucosa and (potentially) total rates of absorption. Neither of the segments where rates of absorption were measured (jejunum and caecum), however, varied in a systematic way.

The 13% increase in daily food intake in response to dilution of a basal diet (0G) with 200 g powdered cellulose/kg (diet 20C, Table 3) is like the 11% increase found in Japanese quail with the same treatment (Savory & Gentle, 1976*a*), both reflecting compensation for inclusion of a practically inert filler. Effects of 200 g cellulose/kg on GI dimensions were not the same in the two species, however, because although their caecal and colo-rectum responses were similar, gizzard weight increased in Japanese quail but not fowl, while small intestine length increased in fowl but not Japanese quail (Table 4; Savory & Gentle, 1976*a*). Increasing the dilution level from 200 to 400 g/kg caused increases in lengths of caeca but not small intestine with both grass and cellulose inclusions, in fowl and Japanese quail respectively (Table 4; Savory & Gentle, 1976*a*).

Compared with the unsupplemented diet 20G, addition of the polysaccharidase enzyme preparation (diet 20G/E) caused an 8% reduction in small intestine length and a 7% increase in caeca length (Table 4), possibly reflecting the 7% improvement in digestibility (Table 3) and increased availability of degraded dietary constituents for absorption or

fermentation in those regions. Similar decreases in small intestine length have also been found in broiler chicks in which digestibility was improved and incidence of sticky droppings reduced by addition of pentosanase and β-glucanase enzymes to certain cereal-based diets (Hesselman & Aman, 1986; Pettersson & Aman, 1989). However, it seems unlikely that such an effect could have occurred here with the diet 20G/E treatment, because, although the enzyme preparation used did contain the appropriate activities, there was no evidence with any of the diets (Table 1) of viscosity of digesta that might have been associated with their cereal component.

The increase in caeca length with diet 20G/E was similar to that caused by diet 40G, indicating increased availability of substrate for caecal fermentation with both treatments. In another experiment with broiler chicks, their caeca showed more evidence of fermentation and were heavier and longer when they were fed on a diet supplemented with 50 g/kg of either arabinose or galacturonic or glucuronic acids, than they were with addition of glucose, galactose or xylose (Longstaff *et al.* 1988). Here, high concentrations of arabinose (a major constituent of hemicellulose) or fermentable oligosaccharides produced by action of the added enzyme could have been the reason for the larger caeca with diets 40G and 20G/E. The mechanism of trophic effects of diet on GI morphology is not known but may, perhaps, involve GI hormones (Lankisch, 1980).

Although absolute recoveries of ^{14}C radioactivity in the liver did not differ significantly between jejunum and caecum perfusions, it can be seen from Table 9 that a considerably greater proportion of the total activity absorbed was recovered in the liver after perfusions in the caecum. Activity was recovered in plasma after jejunum but not caecum perfusions, and total recovery in the whole perfused jejunum segment would have been considerably greater than in the caecum segment because the former was about 2·5 times heavier than the latter (see p. 81). If the apparent differences in the fate of sugars from jejunum and caecum cannot be accounted for by either a difference in the rate at which they were absorbed (and, hence, some threshold effect in the liver) or their conversion to VFA by caecal bacteria (see p. 85), then the implication seems to be that the liver can somehow distinguish and treat differently compounds absorbed in the two regions. It is difficult to envisage how this could be so, since venous blood from both jejunum and caecum enters the liver via the common hepatic portal vein (Nickel *et al.* 1977). Nevertheless, such a conclusion might make sense if most compounds absorbed in caeca, such as VFA and perhaps toxic substances as well, require different treatment anyway in the liver from those absorbed in the small intestine.

This research was funded jointly by a consortium of seven companies and the UK Department of Trade and Industry (Enzymes in Animal Feeds). Thanks are due to them, to Mrs A. Watson for her skilled technical assistance with the work, to Mr J. Culbert for doing the VFA extractions, and to Mrs C. McCorquodale for help with the statistical analysis.

REFERENCES

Bolton, W. & Blair, R. (1974). *Poultry Nutrition*. London: H.M. Stationery Office.

Cheng, B.-Q., Trimble, R. P., Illman, R. J., Stone, B. A. & Topping, D. L. (1987). Comparative effects of dietary wheat bran and its morphological components (aleurone and pericarp-seed coat) on volatile fatty acid concentrations in the rat. *British Journal of Nutrition* **57**, 69–76.

Crane, R. K. (1974). Intestinal absorption of glucose. In *Biomembranes*, vol. 4A, *Intestinal Absorption*, pp. 541–553 [D. H. Smyth, editor]. London: Plenum Press.

Gauthier, A., Larbier, M. & Blum, J. C. (1979). Influence des glucides alimentaires sur la croissance et la vitesse d'absorption intestinale du glucose et du fructose chez le poulet. (Influence of dietary sugars on the growth and rate of intestinal absorption of glucose and fructose in the chicken). *Annales de Biologie Animale, Biochimie et Biophysique* **19**, 703–707.

Gentle, M. J. & Savory, C. J. (1975). The effect of dietary dilution on the intestinal anatomy of the Japanese quail (*Coturnix coturnix japonica*). *Research in Veterinary Science* **19**, 284–287.
Hesselman, K. & Aman, P. (1986). The effect of β-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low or high viscosity. *Animal Feed Science and Technology* **15**, 83–93.
Krebs, H. A. & Henseleit, K. (1932). Untersuchungen über die Harnstoffbildung im Tierkörper. (Research into the urea formation of the animal body). *Hoppe-Seyler's Zeitschrift für Physiologische Chemie* **210**, 33–66.
Lankisch, P. G. (1980). Trophic effects of gastrointestinal hormones. *Clinics in Gastroenterology* **9**, 773–784.
Leopold, A. S. (1953). Intestinal morphology of gallinaceous birds in relation to food habits. *Journal of Wildlife Management* **17**, 197–203.
Levin, R. J. & Mitchell, M. A. (1982). Intestinal adaptations to fasting - use of corrected kinetic parameters to assess responses of jejunal and ileal absorption *in vivo*. In *Mechanisms of Intestinal Adaptation*, pp. 103–110 [J. W. L. Robinson, R. H. Dowling and E. O. Riecken, editors]. Lancaster: MTP Press Ltd.
Levin, R. J., Mitchell, M. A. & Barber, D. C. (1983). Comparison of jejunal and ileal absorptive functions for glucose and valine *in vivo* – a technique for estimating real K_m and J_{max} in the domestic fowl. *Comparative Biochemistry and Physiology* **74**A, 961–966.
Lewin, V. (1963). Reproduction and development of young in a population of California quail. *Condor* **65**, 249–278.
Lis, M. T., Crampton, R. F. & Matthews, D. M. (1972). Effect of dietary changes on intestinal absorption of L-methionine and L-methionyl-L-methionine in the rat. *British Journal of Nutrition* **27**, 159–167.
Longstaff, M. A., Knox, A. & McNab, J. M. (1988). Digestibility of pentose sugars and uronic acids and their effect on chick weight gain and caecal size. *British Poultry Science* **29**, 379–393.
Moss, R. (1972). Effects of captivity on gut lengths in red grouse. *Journal of Wildlife Management* **36**, 99–104.
Moss, R. (1974). Winter diets, gut lengths, and interspecific competition in Alaskan ptarmigan. *Auk* **91**, 737–746.
Nickel, R., Schummer, A. & Seiferle, E. (1977). *Anatomy of the Domestic Birds* (translated by W. G. Siller and P. A. L. Wight). Berlin, Hamburg: Verlag Paul Parey.
Pappenheimer, J. R. & Reiss, K. Z. (1987). Contribution of solvent drag through intercellular junctions to absorption of nutrients by the small intestine of the rat. *Journal of Membrane Biology* **100**, 123–136.
Peddie, J., Dewar, W. A., Gilbert, A. B. & Waddington, D. (1982). The use of titanium dioxide for determining apparent digestibility in mature domestic fowls (*Gallus domesticus*). *Journal of Agricultural Science* **99**, 233–236.
Pendergast, B. A. & Boag, D. A. (1973). Seasonal changes in the internal anatomy of spruce grouse in Alberta. *Auk* **90**, 307–317.
Pettersson, D. & Aman, P. (1989). Enzyme supplementation of a poultry diet containing rye and wheat. *British Journal of Nutrition* **62**, 139–149.
Savory, C. J. (1987). How closely do circulating blood glucose levels reflect feeding state in fowls? *Comparative Biochemistry and Physiology* **88**A, 101–106.
Savory, C. J. & Gentle, M. J. (1976*a*). Effects of dietary dilution with fibre on the food intake and gut dimensions of Japanese quail. *British Poultry Science* **17**, 561–570.
Savory, C. J. & Gentle, M. J. (1976*b*). Changes in food intake and gut size in Japanese quail in response to manipulation of dietary fibre content. *British Poultry Science* **17**, 571–580.
Savory, C. J. & Knox, A. I. (1991). Chemical composition of caecal contents in the fowl in relation to dietary fibre level and time of day. *Comparative Biochemistry and Physiology* **100A**, 739–743.
Savory, C. J. & Mitchell, M. A. (1991). Absorption of hexose and pentose sugars *in vivo* in perfused intestinal segments in the fowl. *Comparative Biochemistry and Physiology* **100A**, 969–974.
Seal, C. J. & Mathers, J. C. (1989). Intestinal zinc transfer by everted gut sacs from rats given diets containing different amounts and types of dietary fibre. *British Journal of Nutrition* **62**, 151–163.
Sudo, S. Z. & Duke, G. E. (1980). Kinetics of absorption of volatile fatty acids from the ceca of domestic turkeys. *Comparative Biochemistry and Physiology* **67**A, 231–237.
Thomas, D. H. & Skadhauge, E. (1988). Transport function and control in bird caeca. *Comparative Biochemistry and Physiology* **90**A, 591–596.

Printed in Great Britain